with 5x magnification and 0.5 numerical aperture enables the generation of high resolution, large field-of-view datasets with imaging depths of several millimeters, sufficient to generate 3D image sets of mouse intestine, heart, lung, brain and other organs.

2988-Pos

The Role of the Protein Matrix in GFP Chromophore Biosynthesis: A Molecular Dynamics Study

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Spontaneous chromophore formation is probably the key feature for the remarkable success of GFPs (Green Fluorescent Proteins) and related fluorescent proteins in bioimaging and fluorescence microscopy. Though a quantitative analysis of the energetics of chromophore biosynthesis still remains elusive, substantial progress has been made [1] in identifying the various steps involved in the reaction and in unveiling the role played by the protein scaffold and by individual residues. A mechanical compression was initially proposed as a determinant factor in triggering backbone cyclization (the first step in chromophore formation) of the chromophore-forming tripeptide [2]. This compression was later ruled out on the basis of X-ray and mutagenesis studies, leading instead to the formulation of a conjugation-trapping mechanism, where the endothermic cyclization product is trapped by subsequent oxidation [3]. Here, by molecular dynamics simulations and potential of mean force calculations, we shall present an estimate of the contribution of the protein scaffold in promoting the proximity of the reacting atoms (a backbone amide and a carbonyl group) - and hence backbone cyclization - by a sort of compression mechanism. Comparing several mutants we shall highlight the role of some residues within or surrounding the chromophore-forming tripeptide. Finally, we shall analyze the case of the HAL (Histidine Ammonia-Lyase) enzyme active site, which undergoes a cyclization reaction analogous to the one in GFP.

[1] R. M. Wachter, Acc Chem Res 40, 120 (2007).
[2] B. R. Branchini, A. R. Nemser, and M. Zimmer, J Am Chem Soc 120, 1 (1998).
[3] D. P. Barondeau, C. D. Putnam, C. J. Kassmann, J. A. Tainer, and E. D. Getzoff, Proc Natl Acad Sci U S A 100, 12111 (2003).

2989-Pos

Metabolism-Excitation Coupling in a Model of K_{ATP} Channel Neonatal Diabetes Mellitus

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The K_{ATP} channel is critical to pancreatic beta cells, linking glucose metabolism with insulin secretion. K_{ATP} channel mutations that reduce ATP-sensitivity lead to neonatal diabetes mellitus (NDM). Expression of these K_{ATP} channel mutations in mice also leads to severe diabetes. Beta cell mass and insulin content are initially preserved, but both decrease with time following the prolonged hyperglycemia. When glycemic control is imposed by transplantation of exogenous islets, this secondary loss of beta-cell mass and insulin content is avoided. We have examined various steps of the glucose stimulated insulin secretion pathway in islets that express ATP-insensitive K_{ATP} channels, which are either unprotected from hyperglycemia or protected by islet transplantation. In protected islets, expression of mutant K_{ATP} channels leads to a severe blunting of glucose stimulated $[Ca^{2+}]$ activity. There remains some sulfonylurea stimulated $[Ca^{2+}]$ and normal KCl stimulated $[Ca^{2+}]$, with insulin secretion following a similar pattern. In unprotected islets, isolated from severely diabetic mice, very similar patterns of $[Ca^{2+}]$ activity are measured but insulin secretion is markedly decreased.

Using two-photon microscopy of NAD(P)H we find glucose metabolism is unaltered in protected mutant islets. This suggests that the reduced $[Ca^{2+}]$ elevation and thus mitochondrial Ca^{2+} uptake has little effect on mitochondrial metabolism. However in unprotected islets, there is elevated basal NAD(P)H and an absence of glucose stimulated NAD(P)H increase, suggesting a disruption of glycolysis and/or mitochondrial metabolism.

Finally the low level of glucose stimulated $[Ca^{2+}]$ in mutant islets is partially synchronized, indicating coupling is present. Reduction of gap junction conductance via chemical inhibitors or islet dispersal leads to elevated glucose stimulated Ca^{2+}. This indicates an important role for gap junctions in regulating Ca^{2+} triggering in the presence of K_{ATP} channel mutations.

2990-Pos

ATP Gradient Across the Innermitochondrial Membrane

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Adenosine 5'-triphosphate (ATP) is one of the most important biological molecules, playing roles as an energy-currency and also as an intracellular and extracellular signal transducer of the cells. In spite of its importance, however, how ATP distribute inside a cell is not clear, because cellular ATP is usually measured after disruption of numbers of cells. Recently, we have developed genetically-encoded Förster resonance energy transfer (FRET)-based indicators specific to ATP, which are called ATeams. These indicators enabled us to monitor ATP concentrations at any desired place inside living cells. To investigate whether there is any biased ATP distribution inside living cells, we compare ATP level of cytoplasm, nucleus and mitochondria using ATeams. ATP levels of cytoplasm and nucleus were almost the same, suggesting that ATP can freely pass through nucleic pores. On the other hand, we observed that ATP level of mitochondria matrix was significantly lower than those of cytoplasm and nucleus. Therefore, there is a gradient of ATP across inner mitochondrial membrane. When the loss of membrane potential of mitochondria was induced by an uncoupling reagent, CCCP, mitochondrial ATP level elevated and the gradient of ATP across the inner mitochondrial membrane disappeared. In addition, loss of membrane potential of mitochondria during apoptosis also resulted in the elevation of mitochondrial ATP. Our results suggest that membrane potential-dependent unidirectional exchange of ATP and ADP by ATP:ADP carrier proteins occurs very rapidly, enough to keep intramitochondrial ATP lower than outside.

2991-Pos

Alpha Hemolysin Induces an Increase of Erythrocytes Calcium: a Fluorescence Lifetime Imaging Microscopt Study

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α-hemolysin (HlyA) from *Escherichia coli* is considered as the prototype of a family of toxins called RTX (repeat in toxin), a series of proteins that share genetic and structural features. HlyA is an important virulence factor in *E. coli* extraintestinal infections, such as meningitis, septicemia and urinary infections. High concentrations of the toxin causes the lysis of several cells as erythrocytes, granulocytes, monocytes, endothelial cells and renal epithelial of different species and low concentrations induces the production of cytokines and apoptosis. Eriptosis, the apoptosis process in erythrocytes, can be induced by several toxins and the increase in calcium concentration inside the cell is being postulated as the trigger of this process. In this context, we followed the calcium concentration inside the erythrocytes while incubating with sublytic concentrations of HlyA; calcium concentration was monitored following the changes in lifetime of the calcium indicator Green 1 using fluorescence lifetime imaging microscopy (FLIM). Data were analyzed using the phasor representation.

In this report we present evidences that, at sublytic concentrations, HlyA induces an increase in Calcium concentration in rabbit erythrocytes in the first 5 minutes. Results are discussed in relation to the difficulties of measuring Calcium concentrations in erythrocytes where hemoglobin is present, the contribution of the background, the heterogeneity of the response observed in different cells and how the phasor approach for lifetime measurements analysis can solve these challenges successfully.

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2992-Pos

Measuring Diffusion Coefficients in Confined Systems Via Multi-Photon Fluorescence Recovery after Photobleaching

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Multi-photon fluorescence recovery after photobleaching (MP-FRAP) is a microscopy technique used to measure the diffusion coefficient of macromolecules in both in vitro and in vivo biological systems. As MP-FRAP is introduced into more systems in vivo, the need arises to expand the technique for application to a wider range of physiological situations. In this poster, we present our investigations into measuring diffusion coefficients via MP-FRAP in bounded systems. We begin by modeling both diffusion and the fluorescence recovery process within a bounded system via Monte Carlo simulations. We then move in vitro, taking and analyzing fluorescence recovery curves in the presence of one and two boundaries. From our results, we determine three limiting cases: 1) boundaries are sufficiently far away to allow the use of MP-FRAP as currently formulated, 2) boundaries are so close as to prevent the use of MP-FRAP entirely, and 3) boundaries are located in a range between these limits, and MP-FRAP is applicable, with modifications to the analysis.